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Chimeras in Mythology and Biology -- How to Overcome the Incompatibility of Tissues -- Transplantation of Blood-Forming Tissue and Radiation Sickness -- New Paths for the Study of the Problem of Transplantation.

In ancient Greek mythology chimeras were monsters with a lion's head, a dragon's tail, and a goat's trunk. However, as such beings were encountered only in legends and could not be seen in reality, the word "chimera" acquired another meaning: it began to be applied to an unreal dream, a fantasy. But we are living in an age in which those dreams which until recently seemed to be the fruits of an unrestrained fantasy are now coming true. This concerns not only the achievements of physics, mechanics, and technology -- as a result of which the Soviet people have created an artificial planet, fired a rocket to the Moon, and photographed the previously unseen side of the latter -- for revolutionary changes have also spread to biology. One example could be the creation of the so-called radiation chimeras.

The problem of the transplantation of the organs and tissues of animals and man is of major importance and has long been attracting the attention of scientists. However, the numerous attempts at solving this problem had not yielded promising results. It was established that the transplantation (grafting) of tissues from an individual of one zoological species to an individual of another, i.e., the so-called heterotransplantation, could not succeed. Moreover, the transplantation of tissues between individuals of the same species -- homotransplantation -- usually also yields negative results. The principal cause for these failures is the immunological incompatibility between the tissues of the host and the donor. The nature of this phenomenon consists in the following. Any tissue contains a multiplicity of antigens -- mostly proteins -- substances which, when introduced into the tissue of an organism of another species cause it to form antibodies capable of binding the given antigens. Upon the transplantation of a heterologous tissue, a reaction directed against the introduced heterologous antigens arises in the organism of the animal to which that tissue is transplanted. As a result of the interaction between antibodies and phagocytes, the

transplanted tissue usually detaches itself 10 to 12 days, or even sooner, after its ingrafting. A widely known example of the antigenic heterogeneity of the tissues of different individuals is the presence of different group antigens in the erythrocytes of human individuals. This makes it possible to conduct blood transfusion usually only for individuals in the same blood group and people of the fourth group (an exception is constituted by the blood of the first group, which contains no corresponding antigens and therefore can be transferred to any human individual).

The experimental studies of transplanting immunity are widely based on pure lines of animals, mostly white mice, obtained as a result of inbreeding. These lines are comparatively homogeneous in the antigen sense, and therefore transplantation within the limits of every pure line (isotransplantation) usually is successful.

Attempts at reducing the immunological reactivity to a transplanted heterologous tissue have been frequently undertaken. However, it was only during the last decade that positive results were obtained in this field. Of particular value are the attempts at reproducing the so-called immunological tolerance.

The theoretical foundation for the work in this field was a hypothesis advanced by F.M. Burnet and F. Fenner (1949, Australia) and, independeltly of them, by V.G. Lopashov and O.G. Stroyeve (1950). According to that hypothesis, animals do not react against a number of antigenic complexes of their own body, because the latter grows together with these complexes and hence becomes tolerant, i.e., resistant to substances which under other conditions would display their antigenic effect. This hypothesis attempts to account for the formation of auto-antigens which may provoke an immunological reaction (in particular, formation of antibodies) in the organism of the same individual in whom they arose.

Viewed from this standpoint, auto-antigens are either substances "maturing" in the process of individual development (ontogeny) after the organism develops an ability to react immunologically, so that the organism no longer can acquire tolerance to their effect; or as antigens forming early but unable, under normal conditions, to penetrate into the sphere of actions of immunological reactions. The first category of auto-antigens includes spermatozoids and, possibly, the milk proteins; and the second -- the protein of the chrystalline lens of the eye and certain antigenic components of the brain.

Proceeding from these premises, the authors of the hypothesis postulated that if some given heterologous antigenic complex is introduced into the organism during its embryonic period, then the host's immune system will gradually adapt itself thereto, and this may result in the formation of a state of immunological tolerance, i.e., inability to react against a given antigen during the post-embryonic period.

The first experimental proofs of these conjectures were obtained by R.E. Billing, L. Brent and P.B. Medavar in 1954. Subsequent investigations corroborated that the introduction of heterologous antigens during the period of embryonic development or in the first few postnatal

does indeed create a state of immunological tolerance. This is particularly successful in the experiments with homotransplantation, and more rarely in those with heterotransplantation.

Certain other methods of artificial suppression of transplantational immunity have also been proposed. However, the procedures on which they are based still continue to yield very unstable results. Even in the experiments in which tolerance to homotransplants is artificially induced during the embryonic period, positive results are far from being always obtained; as for heterotransplantation, in such experiments it succeeds to a known extent only between related species. Moreover, not one of these methods makes it possible to transplant in toto any tissue of an animal onto an animal of another species.

In this connection there arises the question whether this is possible at all? In other words, is it possible artificially to create chimeras, i.e., organisms in which organs and tissues taken from an animal of another species not only acclimatize themselves completely but also replace completely -- morphologically and functionally -- the animal's own corresponding organs and tissues?

Theoretically, such a possibility could hardly be rejected. It is natural to assume that such shimeras can be created only in the event the host's natural immunity to heterologous antigens could be successfully suppressed. Until recently, however, the attempts at materializing this used to founder against imsuperable difficulties relating primarily to the circumstance that even when such a drastic reduction of inborn immunity succeeds, it usually leads to the death of a given organism.

New opportunities for tackling this problem have appeared in the last few years in connection with the intensive studies of the effect of ionizing radiation and, in particular, in connection with its influence on the processes of infection and immunity. At present it has been firmly established that exposure to medium and large doses of ionizing radiation can cause a drastic decrease of inborn immunity in a number of pathogenic (disease-inducing) and even nominally pathogenic microorganisms, which is partly related to a sharp drop in the production of antibodies upon radiation injury.

Moreover, it was found that radiation sickness is accompanied by a steep decrease in inborn immunity also with regard to the immunity to the heterologous tissues of ther multicellular organisms. However, this state is comparatively short in duration, lasting on the average three or four weeks. Upon the elapse of that period, as a rule, the organism's reactivity renews itself and the transplants detach themselves.

How to overcome this difficulty? The reply was obtained unexpectedly. New experimental opportunities appeared upon studying a problem which seemingly bears no direct relation to this matter. We are speaking of the therapy of radiation sickness by means of biological preparations. At first glance, this circumstance seems strange.

In effect, if radiation sickness reduces transplantational immunity then it should be expected that a successful therapy of radiation sickness should, conversely, be conducive to the restoration of the inborn immunity which was upset by irradiation, whereas a successful transplantation requires the suppression of that immunity. However, it is precisely the experiments in the biotherapy of radiation injuries that have yielded new facts which made it possible to reexamine the problem from new standpoints and which led to the creation of the so-called radiation chimeras.

It was found that the intravenous administration of splenic tissue suspension to irradiated animals or the transplantation of the spleens of normal, nonirradiated animals to irradiated animals results in a therapeutic effect (Jacobson and co-workers, N.I. Shapiro and co-workers, A.G. Karavanov and co-workers, and others) expressed most clearly in the experiments with isotransplantation (between animals of one and the same pure line); and less clearly on introducing a suspension of splenic cells belonging to an animal of the same species. The least graphic but nevertheless not less distinct results were obtained from a series of experiments with the heterotransplantation of spleen.

Somewhat later it became possible to establish the therapeutic effect of a suspension of bone marrow cells on radiation sickness, as manifested most explicitly upon their intravenous administration within the first four days after radiation exposure (L.I. Koul and co-workers, N.I. Shapiro and co-workers, V.L. Troitskiy and M.A. Tumanyan, and others). Here the therapeutic effect was also achieved in introducing both the isologous and homologous and the heterologous bone marrow. In connection with these researches, which are of major significance to the theory and practice of the struggle against radiation sickness, there arose the question of how to explain the high therapeutic effect of the blood-forming tissue when it is introduced into an irradiated organism.

It is known that the earliest symptoms of radiation sickness are the injury of the lymphoid elements of the spleen and the lymph nodes, and sells of bone marrow as well. In this connection, in the irradiated animals into which cells of the spleen or bone marrow are introduced, the destroyed cells of the blood-forming tissue usually are rapidly restored. What causes this, the self-reproduction of the host's cells or that of the donor's cells?

In this connection, two opinions have been offered. Some investigators postulated that this stems from the presence in the blood-forming organs of the nonirradiated animals of a special factor stimulating blood formation (in a number of cases favorable results could be achieved when introducing the cell-free filtrates of blood-forming organs into irradiated animals). It was also postulated that the introduced cells reproduce in the organism of the irradiated host. The experiments arranged for the purpose of corroborating this hypothesis have led to extraordinarily interesting results.

First of all, it was necessary to develop methods which would make it possible to detect the self-reproduction of cells introduced into the irradiated organism. As is known, during the microscopy of the histological sections of tissues usually it is not possible to detect distinct differences between the cells of the blood-forming tissue of the various species of mammals; it is not possible, a fortiori, to detect such differences when comparing the cells of various individuals of a single species. However, the inquiring spirit of researchers has found paths for overcoming these difficulties.

Four methods were developed for establishing the presence of the donor's cells in the host's organism: histochemical, cytological, immunological, and physicochemical.

The histochemical method has made it possible to detect very explicitly the presence and multiplication of the granulocytes of rats in the organisms of mice. The basis of this method is that the granulocytes of rats contain a much greater amount of alkaline phosphatase than do the analogous cells of mice, and that this manifests itself in their differing colors when definite reagents are applied to the tissue.

The cytological method was utilized, in particular, in the experiments of K.E. Ford and his co-workers (1956); they used for this purpose a semisterile strain of mice, T-6, in which one of two chromosomes is always smaller than the pairing chromosome during the metaphase, and has a characteristic shape. Upon introducing the splenic cells from the mice of that strain into irradiated mice of another pure line, it was possible in every case to detect these small chromosomes-markers in the splitting cells of bone marrow and spleen, lymph nodes, and thymus of the host. J. Trentin (1956) in his experiments utilized the cytological peculiarities of the neutrophils* of rabbits and guinea pigs, which have characteristic granules distinguishing them from the neutrophils of mice.

The immunological methods make it possible to detect the donor's cells in the transplant-receiving organism by means of various immunological reaction tests. The physicochemical method makes it possible to establish the presence of heterologous erythrocytes in the organism of the irradiated host.

By means of the above-enumerated methods it was established that the cells of the donor's blood-forming tissue, when introduced into the irradiated host, are capable of reproducing themselves in the latter's organism. In other words, it became possible to obtain a valid transplantation of heterologous blood-forming tissue. Of special interest is the fact that such results were obtained not only in experiments with iso- and homotransplantation but also in a number of experiments with the grafting of heterologous blood-forming tissue (cells of bone marrow or spleen) onto irradiated animals.

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* Neutrophils are white blood cells stained by both acidic and basic pigments.

The effectiveness of the transplantation of heterologous blood-forming tissue onto irradiated organisms hinges to a major extent on the genetic affinity between the donor and the host. The best results are obtained by the heterotransplantation of blood-forming tissue from a related species. This, apparently, may explain the fact that in a majority of cases in such experiments mice served as the irradiated animals (hosts) and rats, as the donors. It is, however, to be kept in mind that such a choice of animals is convenient also in the sense that it is comparatively easy to establish the presence of the cells of the blood-forming tissue of a rat, expecially granulocytes, in the organism of a mouse.

The experiments conducted by a number of researchers during the last two or three years make it possible to arrive at the conclusion that upon introducing heterologous blood-forming tissue into irradiated hosts it is possible, under specific conditions of experiment, to obtain true radiation chimeras in which the introduced tissue is capable of not only reproducing itself but also supplanting the host's analogous tissue. Such results are, as a rule, obtained in the case of exposing the host to an absolutely lethal dose of ionizing radiation. Thus, e.g., in the experiments of M. Zhengozyan and T. Makinodan (1957, United States), the introduction of bone marrow from nonirradiated rats into mice irradiated with a dose of 710 roentgens, which had caused the death of 30 percent of the animals, not only did not increase the survival rate but also, on the contrary, promoted the death of the irradiated mice. The maximal therapeutic effect of the introduction of bone marrow was obtained in application to mice irradiated with a dose of 950 roentgens. In all the mice surviving for more than 150 days after irradiation with 950, 1,150 and 1,300 roentgens, all erythrocytes were of rat origin. Apparently, a maximally successful heterotransplantation of bone marrow requires the exposure of the host to high doses of ionizing radiation causing a massive destruction of the host's own blood-forming tissue and suppressing drastically the immunological reactivity of the host's irradiated organism, so that as a result the host no longer can reject the introduced heterologous tissue.

Radiation chimeras are chimeras not only in the morphological but also in the immunobiological sense. Their nature changes radically, approximating that of the donor. Thus, for example, the irradiated mice in which the introduced bone marrow of rats has replaced a major part or all of their own blood-forming cells begin to react to the introduction of various antigens not like mice but like rats. This striking phenomenon manifests itself with special clarity in experiments dealing with skin grafting. Thus, in the experiments of O.B. Zalberg, O. Vos and D.V. Bekk (1957), irradiated mice were protected from radiation sickness by introducing the bone marrow of a pure line of rats, "Wistar Albino Claxo". The 35 surviving mice were, after 24-151 days from the date of their irradiation, given grafts of skin pieces from rats of the same strain.

A normal growth and prolonged adaption of the graft were observed in all the 10 mice in which all granulocytes* and erythrocytes were of rat origin. In nine of the other cases, the growth of the graft was irregular -- both mose and rat granulocytes were detected in the blood of these mice. In three animals, in whose blood no cells of the rat type were detected, complete rejection of the graft was observed. The remaining 13 mice perished, but all with viable grafts at the moment of their death. In the control experiments with the grafting of rat skin onto 37 non-irradiated mice, all animals were observed to reject the grafts within not later than 12 days after they were made.

Thus, when heterologous blood-forming tissue is introduced into irradiated animals, it is not only capable of reproducing itself in the host's organism and replacing the latter's own blood-forming tissue but such an interference results also in a prolonged specific change in the immunological properties of the hose, as expressed in the development of the state of tolerance in the host, i.e., in the suppression of immunological reactions against the transplants of tissues from the strains or species of animals whose blood-forming tissue is introduced into irradiated hosts. The degree of this tolerance apparently does not depend on the degree of heterologousness of the introduced cells but hinges directly on the ability of these cells to reproduce themselves in the organism of the irradiated host: the more completely the introduced cells of blood-forming tissue replace the analogous cellular elements of the irradiated host, the more distinctly expressed is the latter's inability to reject a heterologous tissue (in particular, skin) transplanted onto him.

It should, however, be noted that when a homologous or heterologous blood-forming tissue is introduced into an irradiated host then, despite the adaption of the introduced cells and the distinct decrease in the mortality of the irradiated organisms during the first 20 days after their irradiation, a part of the animal dies in the subsequent periods: within 21 to 120 days after irradiation. This so-called secondary sickness, causing the late (delayed) death of irradiated animals, is not observed in isltransplantation. A majority of the investigators assume that the delayed mortality is a result of the antigen-antibody reaction. According to one standpoint, antibodies are produced by the organism of the irradiated host; it is assumed that they react with the antigens of the introduced blood-forming tissue, which also is the reason for the onset of secondary sickness. Other investigators postulate that the introduced blood-forming elements, on reproducing themselves and replacing the blood-forming tissue of the irradiated organism, themselves produce antibodies directed against the host's tissues. Both these hypotheses are based on definite experimental data, and as yet it is difficult to

* White blood cells with protoplasm -- having granular structure.

decide which one is more justified. Apparently, both these possibilities are quite real, but the degree of manifestation of each depends on concrete conditions in every individual case. One such condition is, in particular, the amount of the host's immunogenetic tissue remaining undamaged after the irradiation, and also the extent of its regeneration.

So that the donor's cells could exist in a genetically different (heterologous) irradiated host, the antigenically different tissues of the hosts and the donor should be subject to mutual adaptation in such a way as to cease to react to one another. In radiation chimeras the regeneration of the host's tissues after irradiation develops in the presence of the blood-forming tissue of the donor. On the other hand, during the self-reproduction of the donor's cells in the organism of the irradiated host, these self-reproducing cells may also adapt themselves to the host's tissues and become tolerant to them.

Secondary sickness develops at incomplete adaptation or in the absence of that adaptation because of a chronic antigen-antibody reaction. In isotransplantation there occurs a total mutual adaptation between the tissues of the host and the donor, the antigen-antibody reaction does not arise, and secondary sickness does not develop. In the final analysis, the process of mutual adaptation depends on other factors: on the degree of regeneration of the host's immunogenetic tissue and on the degree of self-reproduction of the immunogenetically active cells of the introduced blood-forming donor tissue.

Thus, the feasibility of obtaining radiation chimeras under experimental conditions can be regarded as a definitely established fact. In this connection, the researchers concerned with the problems of the transplantation of organs and tissues are facing newly revealed prospects. At present the practical application of blood-forming tissue for the treatment of radiation sickness is complicated by the circumstance that upon the introduction of homologous or heterologous bone marrow (or spleen) a major part of the irradiated hosts perishes within 21 to 120 days after irradiation as a result of secondary sickness.

The most important task in this field, therefore, is to develop methods for the prophylaxis of delayed mortality in the homologous and heterologous radiation chimeras. Certain encouraging results have already been attained in this direction. Thus, e.g., A. Lengerova (1957, Czechoslovakia) established that, in contrast with the homotransplantation of the blood-forming tissue from adult animals, which results in delayed mortality, the latter is not observed when cells of embryonic blood-forming tissue are introduced into irradiated animals.

Recently the following event had happened. On 15 October 1958, in Yugoslavia, as a result of the breakdown of an atomic reactor, six persons were exposed to radiation, five of them receiving absolutely lethal doses of neutron and gamma radiation (from 600 to 1,200 roentgens). The victims were flown to Paris, where homologous bone marrow was infused into the five who had received lethal doses of radiation. Successful immediate results were obtained for four of the five patients;

bone marrow collected by puncturing the bones of adult donors of the same blood group as the victims was administered to the victims. An emulsion of bone marrow amounting to 183-300 milliliters (8-14 billion cells) was infused into the victims on the 24th, 33rd, and 36th days after radiation injury. Special investigations established that a prolonged adaption of the introduced cells proceeded in the organism of the patients. In all the four surviving patients a clearly expressed increase in the count of white and red blood cells, gain in weight and improvement in general state had been observed as soon as after 48 hours from the time of infusion.

These data were published in March 1959 by the French scientists G. Mate, A. Zhamme, and others. Considering that that catastrophe had happened on 15 October 1958, it can be assumed that these favorable results did not abate for at least four months since the radiation injury. We cannot as yet speak of the ultimate results. In particular, the possibility of the development of a secondary immunological reaction (secondary sickness at a later time) is not excluded. However, the fact that the patients are still alive serves as the proff of a prodigious feat of modern biology and medicine.

This provides a foundation for the hope that in the immediate future it will become possible to find effective means for combatting secondary sickness, which will make it possible, in turn, to use suspensions of cells of homologous and heterologous blood-forming tissue for the treatment of radiation sickness. The enormous theoretical importance of the work on the artificial creation of radiation chimeras is indisputable. This research is opening up new paths for the study of the mechanism of transplantational immunity, and it makes it possible to consider the problem of the heterologousness of species from new theoretical standpoints.

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